Supplement

Stimulant drug effects on touchscreen automated paired-associates learning (PAL) in rats

Corinna Roschlau, Angeline Votteler & Wolfgang Hauber

Department Animal Physiology

University of Stuttgart, D-70550 Stuttgart, Germany

Methods

Subjects

Male Lister Hooded rats (Charles River Laboratories, UK) were housed in groups in transparent plastic cages (60 cm × 38 cm × 20 cm, Tecniplast, Milan) in a 12:12-h light-dark cycle with lights on at 7 AM. All experiments were conducted during the light phase, temperature (22 ± 2 °C) and humidity (50 ± 10 %) were kept constant. Animals had *ad libitum* access to water and were supplied with standard laboratory chow for rodents (Altromin, Lage, Germany) *ad libitum* for the first 7 days after arrival. Thereafter, laboratory chow was restricted to 15 g per animal and day to maintain them on approximately 85 % of their free-feeding weight. For environmental enrichment, a plastic tube (20 cm, Ø 12 cm) was fixed at the lid of each cage and nesting material was provided.

Apparatus

Operant boxes (Med Associates, Vermont USA; 31.8 cm x 25.4 cm x 26.7 cm) were employed with one end of the chamber equipped with a touch-sensitive, flat-screen LCD monitor equipped with an infrared sensor. A black aperture plate (29 x 19 cm) was attached in front of the monitor made of stainless steel with 3 rectangular response windows (6 cm x 9 cm; lower edge 4 cm above box grid floor). The plate was attached to the monitor leaving a 0.5-cm space between the plate and monitor. A shelf (6 cm x 20.5 cm) was attached above the grid floor. The wall opposite the LCD monitor was equipped with a food magazine with a built-in magazine light and head entry detector. Above the food

magazine there was a house light (3 W). Each operant box was kept in a sound-attenuating chamber. The testing apparatus was controlled by the K-Limbic software (Med Associates, Vermont, USA).

Task and training

Animals were trained and tested on the dPAL task (*"different Paired Associate Learning"*) according to a protocol by Talpos et al. (2009) generally on 5 days per week.

Habituation. In brief, animals were placed in the operant chambers for 2 subsequent days for 20 min each to habituate them to the boxes baited with food pellets (45 mg Dustless Precision Pellets #F00231-J, Bio-Serv, Frenchtown, USA) scattered throughout the chamber.

Pretraining: Next, we trained animals to pick up food pellets from the magazine. On Day 1, for 100 trials, the magazine light was switched on upon pellet delivery (every 30 s) and switched off after a head entry into the magazine. On Day 2, white squares were presented in all three of the response windows. If the animal did not touch the monitor, a one-pellet reward was always given after 30 s, if the animal touched the monitor, a three-pellet reward was given on top. Then, the next trial was initiated; the session ended after 100 trials or 30 min whichever came first.

Subsequently, rats were required to touch any area of the monitor to earn a reward. On Day 3 and 4, one session was given per day, a session ended after 100 trials or 30 min whichever came first. Again, white squares were presented in all three of the response windows. The screen remained active until a response occurred. Once a response occurred, one food pellet was delivered and the touchscreen deactivated. The next trial began 5 s after the pellet was collected.

Next, to avoid a development of a response bias, in daily sessions on Days 5 and 6 (including 99 trials each), one of the three response locations was randomly illuminated and the rat was required to poke at this location to earn a food pellet. Pokes at other locations had no programmed consequences.

PAL task training. As shown in Fig. S1, the following symbols were used as stimuli on the touch screen: flower, plane and spider. Each symbol was correct (S+) in one particular position on the screen (left (1), middle (2) or right (3)) and incorrect in the other two positions (S-). Correct positions for each symbol differed within two subgroups of animals. In a given trial always two stimuli were given, one stimulus in the correct (S+), the other in an incorrect position (S-). There are 6 possible trial types;

a session consisted of 72 trials in total, i.e. each trial type was given 12 times in a semi-random manner. A trial of a session began when the rat nose-poked at the illuminated food magazine, a response that caused magazine light deactivation and S+ and S− display upon the screen. A response at the S+ would trigger the delivery of a reward pellet, illumination of the receptacle and cause the screen to go blank. The collection of the reward pellet caused deactivation of the magazine light and initiation of the inter-trial interval (ITI; 10 s). After 10 s, the magazine was re-illuminated and a nose poke to it initiated the next trial. If a rat responded at the S−, a 10-s timeout was initiated, stimuli were immediately removed from the screen, and the house light was switched off for 10 s. After 10 s, the food magazine was re-illuminated, and a nose poke to it triggered a "correction trial". Within a correction trial, the S+ and S− were displayed as in the previous trial. For 2 days, task training included up to 5 correction trials at maximum for a given trial type, on the subsequent day task training included 3 correction trials at maximum. Thereafter, the PAL full task was implemented which had the same design without correction trials. All rats were able to complete 72 trials within 60 min regardless whether correction trials were given or not.

Drugs

Amphetamine (Sigma, Taufkirchen, Germany) was dissolved in saline (0.9% NaCl, Braun Melsungen, Germany) and administered i.p. 30 min pior behavioral testing at a volume of 1 ml/kg. IP injections of saline served as controls. Modafinil ((2-(diphenylmetyhl)sulfinyl)acetamide) purchased from Sequoia (Pangbourne, UK) was dissolved in 1% w/v methylcellulose (Sigma, Taufkirchen, Germany) in saline and administered i.p. 30 min prior behavioral testing at a volume of 2 ml/kg i.p. IP injections of 1% w/v methylcellulose (Sigma, Taufkirchen, Germany) in saline (2 ml/kg) served as controls. Methylphenidate (Sigma, Taufkirchen, Germany) was dissolved in saline and administered i.p. 30 min prior behavioral testing at a volume of 1 ml/kg. IP injections of saline served as controls. MK-801 (Bristol, UK) was dissolved in saline and administered i.p. 30 min prior behavioral testing at a volume of 1 ml/kg. IP injections of saline served as controls.

Experimental procedures

For all experiments, the same group of animals was used (n=6). Animals were given a single vehicle injection 1 week prior to the beginning of testing to habituate them to the injection procedure. All experiments used a within-group design in which each rat received all drugs and respective vehicle treatments with one treatment on one test day per week. Baseline training sessions without drug administration were conducted 4 days per week. The order of drug testing was as follows: amphetamine, methylphenidate, modafinil, MK-801.

Experiment 1: Effects of the amphetamine were assessed. On weeks 1 and 2, a low dose of amphetamine (0.4 mg/kg) was tested *vs*. vehicle using a within-subject cross-over design, i.e. half of the animals received drug or vehicle on the test day in week 1; this assignment was reversed on the test day in week 2. On weeks 3 and 4, a high dose of amphetamine (0.8 mg/kg) was examined using the same experimental design.

Experiment 2: Effects of methylphenidate (4.5, 9 mg/kg, i.p.) were assessed as described for Experiment 1.

Experiment 3: Effects of modafinil (32, 64 mg/kg, i.p.) were assessed as described for Experiment 1.

Experiment 4: Effects of MK-801 (0.8, 0.12 mg/kg, i.p.) were assessed as described for Experiment 1.

Data analysis

Data were analyzed with the Microsoft® Excel-based program "Visual Discrimination Task Analysis Package" (Conclusive Marketing Ltd, Sawbridgeworth, UK). Percent correct, response latencies (time from symbol activation on the monitor to the response at the monitor) and magazine latencies (time from the response at the monitor until magazine entry) are given as means ± standard error of the mean (SEM). Data from each drug dose and respective vehicle control were subjected to a paired t-test. All statistical computations were carried out with STATISTICA TM (version 7.1, StatSoft®, Inc., Tulsa, OK, USA). The level of statistical significance (α-level) was set at $p \leq 0.05$ (α-levels > 0.05 were designated as n.s., not significant).

Supplementary Results

Correct choices across trial categories.

Detailed consideration of the rat PAL task (Fig. S1) shows that correct choices in a subset of trials (termed here "unique-configuration trials", i.e. trials 1 and 6 in Fig. S1, left panel) may exclusively rely on correct object-location associations (i.e. *"flower is always correct on the left position"*/trial 1; *"airplane is always correct on the right position"*/trial 6). By contrast, correct responses in another subset of trials (termed here "common configuration trials", i.e. trials 2-5 in Fig. S1, left panel) could also involve an alternative strategy, i.e. conditional rules based on the configuration of both presented objects.

To investigate the possibility that correct choices in vehicle-treated rats differed in unique *vs.* common configuration trials, we analyzed correct responses from 8 test sessions with vehicle administration (vehicle control sessions each for drug/dose treatment shown in Figs. 1-3 and Fig. S2) as a function of trial category. Note that, to control for location-object preferences, we used two subgroups of rats with different location-object assignments: Fig. S1 left and right panels show 6 different trial types with correct location-symbol pairings used for each subgroup, respectively. Thus, trials 1 and 6 (left panel) and trials 4 and 6 (right panel) represent unique-configuration trials, all other trials are common configuration trials.

Comparison of correct choices in common *vs.* unique configuration trials in each vehicle session revealed significant differences in 3 out of 8 test sessions (Tab. S1). Moreover, we analyzed correct choices in common *vs.* unique configuration trials over the entire experimental period in the last baseline training days (i.e. days immediately prior to the weekly vehicle/drug test day). Data consistently revealed moderately lower correct choices in unique relative to common configuration trials (data not shown). However, a repeated measures ANOVA demonstrated no effects of trial category (unique *vs.* common configuration, ANOVA $F_{1,10}=2.21$, n.s.), an effect of day (ANOVA $F_{15,150}=1.95$, $p<0.05$) but no category x day interaction (ANOVA $F_{15,150}=0.73$, n.s.).

Treatment effects on correct response rates across trial categories.

We also analyzed the possibility that effects of amphetamine, methylphenidate, modafinil and MK-801 on correct choices differ as a function of trial category. To this end, we compared, for each drug/dose combination depicted in Figs. 1-3 and Fig. S2, correct choices in unique configuration trials in the vehicle session and the corresponding drug session; correct choices in common configuration trials were compared correspondingly. Results demonstrated that methylphenidate (9 mg/kg) selectively reduced correct choices in common configuration trials (t_5=3.78; p<0.02, paired *t*-test). Likewise, MK801 (0.12 mg/kg) selectively reduced correct choices in common configuration trials (t_5=2.74; p<0.05, paired *t*-test). Other comparisons were not significant.

Acknowledgements

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References

Talpos JC, Winters BD, Dias R, Saksida LM, Bussey TJ. 2009. A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: a translational rodent model of cognitive impairments in neurodegenerative disease. Psychopharmacology (Berl) 205: 157-168.

Tab. S1. Comparison of correct choices (mean ± SEM) in common (COM) *vs.* unique (UNI) configuration trials in sessions with vehicle administration (depicted in Figs. 1-3, S2)

Vehicle session	Trial type	Mean ± SEM (%)	*t*-test
Amphetamine (0.4 mg/kg) control (see Fig.1)	UNI	75.69 ± 6.49	$t_5 = 1.70$; n.s.
	COM	79.51 ± 5.88	
Amphetamine (0.8 mg/kg) control (see Fig. 1)	UNI	59.03 ± 9.88	$t_5 = 1.39$; n.s.
	COM	70.83 ± 5.19	
Modafinil (32 mg/kg) control (see Fig.2)	UNI	59.72 ± 6.33	$t_5 = 2.54$; n.s.
	COM	75.0 ± 5.05	
Modafinil (64 mg/kg) control (see Fig. 2)	UNI	61.81 ± 6.22	$t_5 = 2.56$; n.s.
	COM	77.08 ± 2.47	
Methylphenidate (4.5 mg/kg) control (see Fig. 3)	UNI	62.50 ± 6.63	$t_5 = 3.14$; $p < 0.05$
	COM	78.82 ± 4.08	
Methylphenidate (9 mg/kg) control (see Fig. 3)	UNI	61.81 ± 6.75	$t_5 = 1.95$; n.s.
	COM	76.04 ± 6.58	
MK801 (0.08 mg/kg) control (see Fig. S2)	UNI	65.97 ± 7.56	$t_5 = 5.27$; $p < 0.005$
	COM	79.17 ± 5.49	
MK801 (0.12 mg/kg) control (see Fig. S2)	UNI	65.97 ± 8.70	$t_5 = 3.02$; $p < 0.05$
	COM	86.46 ± 4.26	

Fig.S1

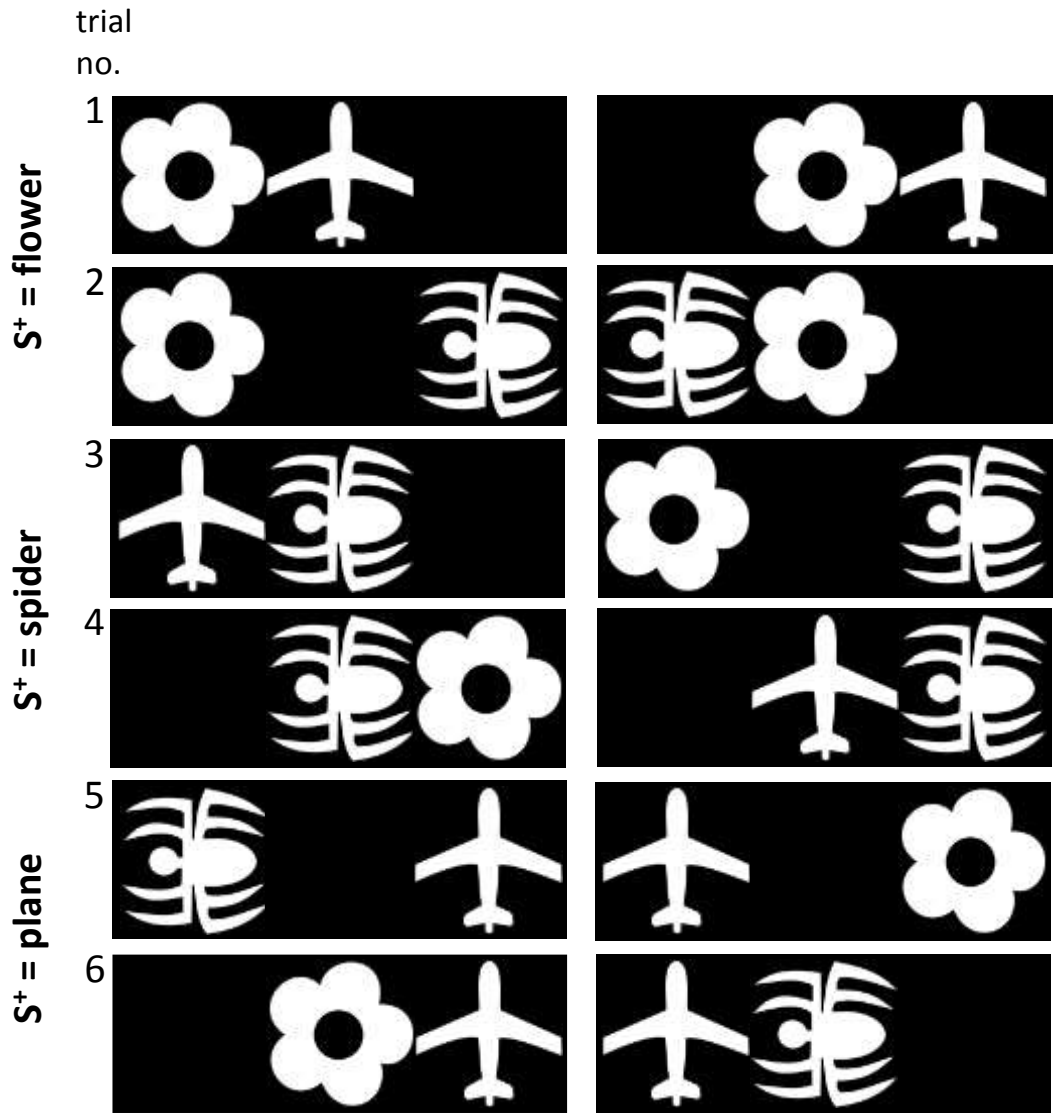

Fig.S1. Illustration of the 6 different trial types with correct location-symbol pairings used for two subgroups of rats, respectively (left, right panel). The correct location for the symbols flower, spider, plane is designated by S+.

Fig.S2

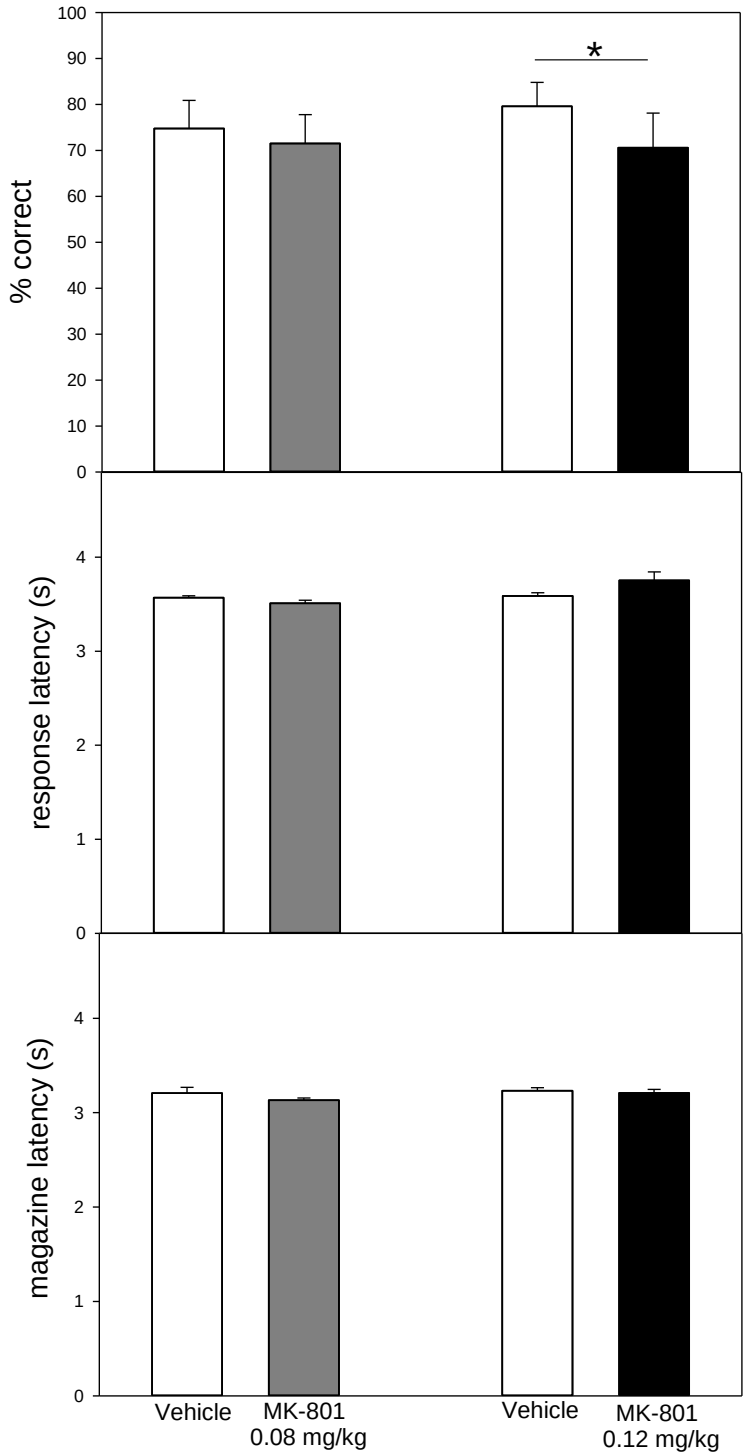

Fig.S2. Effects of MK-801 (0.8, 1.2 mg/kg) on PAL performance. Per cent correct responses, response latencies and magazine latencies are given as means ± SEM. The higher drug dose significantly decreased % correct responses (*, $p<0.05$, paired t-test). No other significant effects were detected.